REMARKS

By the present Amendment, Applicants have canceled pending Claims 46-59 in favor of newly added Claims 60-63. Claims 46-59 are canceled without prejudice or disclaimer as to the subject matter contained therein. Applicants reserve the right to file continuation and/or divisional applications on all or a portion of the subject matter which was canceled by this Amendment.

Applicants present the instant Amendment in conjunction with a Renewed Request By Applicants For Interference Pursuant to 37 CFR § 1.607, wherein Applicants respectfully request that an interference be declared between the above referenced application and U.S. Patent No. 6,174,673. The information required by 37 CFR § 1.607(a) is set forth under headings which correspond to the subsections of § 1.607 to facilitate consideration by the Examiner.

REQUEST FOR INTERFERENCE

I. IDENTIFICATION OF THE PATENT WHICH INCLUDES SUBJECT MATTER WHICH INTERFERES WITH THE APPLICATION

The patent which claims subject matter which interferes with subject matter claimed in the present application ("the present application") is U.S. Patent No. 6,174,673 ("the Short patent") issued on January 16, 2001 to Jay M. Short *et al.* for "High throughput screening for novel enzymes". The Short patent was issued on application Serial No. 09/098,206, filed

January 16, 1998, which purports on its face to be a continuation-in-part of 08/876,276, filed on June 16, 1997. Diversa Corporation is the assignee named on the face of the patent.

II. PRESENTATION OF A PROPOSED COUNT

Attached **Appendix B** sets forth the proposed Count. The proposed Count recites new Claim 60 of the present application after consideration of the subject matter claimed by the respective parties. As required by 37 CFR § 1.601(f), the proposed Count "defines the interfering subject matter between . . . one or more applications and one or more patents."

The Count is proposed as Claim 60 of the present application as it corresponds to claim 8 of the Short patent. Claim 60 includes all the limitations recited in Claim 1 of the Short patent upon which Claim 8 depends. Thus, Claim 60 recites the limitations of Claim 1 and Claim 8 of the Short patent in one claim. The interfering subject matter between Iverson and Short relates to methods for identifying polypeptides using high throughput screening of genomic DNA from mixed populations of organisms.

III. IDENTIFICATION OF CLAIMS OF THE SHORT PATENT WHICH CORRESPOND TO THE PROPOSED COUNT

Claims 1-23 of the Short patent are believed to correspond to the proposed Count. Claims 1 and 22 are independent claims. Claim 8, which depends from Claim 1, is therefore a species of Claim 1. Claim 8 of the Short patent is directed to a method of identifying

bioactivities or biomolecules using high throughput screening of genomic DNA of extremophiles.

Claim 22, although an independent claim, is a species of Claim 1. Claim 22, like Claim 1 of the Short patent, is directed to a method for identifying bioactivities or biomolecules using high throughput screening of genomic DNA, but is limited to a method wherein the genomic DNA is prokaryotic DNA, and wherein the method is performed in a culture-independent manner. The culture-independent method of Claim 22 would also function with extremophiles, as recited in Claim 8 and the proposed Count. Thus, the method of Claim 22 is a species of the method of Claim 21. Claim 23 is dependent on Claim 22 and corresponds to the Count for the same reasons as set forth for Claim 22.

In order to assist the Examiner, attached **Appendix C** sets forth a side-by-side comparison of Claims 1-23 of the Short patent with the proposed Count.

IV. CLAIMS OF THE PRESENT APPLICATION WHICH CORRESPOND TO THE PROPOSED COUNT

Previously pending Claims 46-59 have been canceled without prejudice or disclaimer as to the subject matter contained therein. New Claims 60-63 have been added. New Claims 60-63 are believed to correspond to the proposed Count. To assist the Examiner in this regard, Applicants attach **Appendices A** and **D**. **Appendix A** is a chart providing an element-by-element recitation of the newly added claims of the present application, and an indication of the

passages in the originally filed application[1] where, at the very least, the claims find support. **Appendix D** is a chart providing a side-by-side comparison of new Claims 60-63 of the present application with the proposed Count, which explains the rationale for including these claims in the interference based on the proposed Count.

V. 35 U.S.C. § 135(b) IS SATISFIED

At least one claim was submitted[2] in the above-referenced application which is the same as, or for the same as or substantially for the same subject matter as, a claim of the Short patent, and such claim is being made prior to one year from the date on which the Short patent was granted (*i.e.*, January 16, 2001). Thus, § 135(b) is satisfied.

VI. CONCLUSION

Applicants respectfully request that an interference be declared employing the proposed Count set forth on attached Appendix A, with Claims 1-23 of the Short patent and Claims 60-

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[1] The present application was filed February 12, 2001, and is a continuation/ divisional of U.S. Serial No. 08/847 (hereinafter "the '063 application"), filed on May 1, 1997. The Short patent was filed January 16, 1998, and purports to be a continuation-in-part of Serial No. 08/876,276, filed June 16, 1997. Accordingly, even if Short is accorded benefit of its earliest filed application, Iverson should be designated Senior Party in the interference.

[2] With the Amendment dated August 2, 2001.

63 of the present application designated as corresponding the Count. Such action is respectfully requested.

Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact Applicants' undersigned representative to expedite prosecution.

Respectfully submitted,

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APPENDIX A

New Claim Chart

New Claims 60-63	Support in the Specification[1]
60. A method for identifying polypeptides having a desired activity	p. 1, l. 14 ["identify specific polypeptides having desirable characteristics"]; p. 3, l. 7 ["antibodies with particular binding functions, as well as other activities"]; p. 4, l. 18 ["biocatalyst"]; p. 5, l. 16 ["enzyme activity"]; p. 11, ll. 21-22 ["biologically active polypeptides, in terms of both function and specificity"]; p. 15, ll. 17-19 ["to provide a general approach for the efficient screening of very large libraries of virtually any polypeptide for desirable activity"]; p. 23, l. 19 ["Cells exhibiting the desired activity will be isolated"]; and Claim 4 as originally filed.

[1] This support is exemplary only; support is not intended to be limited to that disclosure specifically referenced. Applicants reserve the right to refer to other passages should they deem such to be desirable.

New Claims 60-63	Support in the Specification[1]
using high throughput screening	p. 7, l. 18 - p. 8, l. 4 ["rapid and efficient screening"]; p. 12, ll. 8-9 ["screened by brute force approaches that rely heavily on robotics"]; p. 15, ll. 17-19 ["to provide a general approach for the efficient screening of very large libraries of virtually any polypeptide for desirable activity"]; p. 32, l. 10 ["high throughput"]; p. 54, ll. 24-25 ["A particularly preferred method for identification and isolation is cell sorting or flow cytometry. One aspect of this method is fluorescence activated cell sorting"]; p. 62, ll. 1-19; p. 64, ll. 27-28 ["rapid measurement of large numbers of individual microcolonies by flow cytometry"]; *see also*, material incorporated by reference at p. 12, l. 9 ["an accelerated robotic screening system that handles several different enzyme assays simultaneously and operates 24 hours a day"]; and Claims 22-24, 35-37 as originally filed.
of genomic DNA comprising:	Support can be found throughout the specification for DNA; *see also* material incorporated by reference at p. 12, l. 9 ["genomic gene expression libraries"].
a) providing an expression library containing a plurality of clones,	p. 54, ll. 3-4 ["a library of cell surface displayed proteins is prepared"]; p. 61, ll. 22-23 ["a cell population where each cell displays a different polypeptide"]; *see also*; and Claim 11 as originally filed.
wherein the DNA for generating the library is obtained from a mixed population of organisms,	See material incorporated by reference at p. 12, l. 8 ["mix of organisms"].

New Claims 60-63	Support in the Specification[1]
wherein the organisms are extremophiles;	p.3, l. 24 ["isolation of thermostable variants"]; p. 12, ll. 4-6 ["cloning and expression of enzyme libraries from organisms that cannot be cultivated and typically are isolated from extreme environments"]; *see also*, material incorporated by reference at p. 12, l. 5 ["extremophiles"].
b) enclosing a fluorescent substrate and at least one clone from the library	p. 67, ll. 12-22 ["the enzymatic reaction with substrate is carried out in AGM's with enclosed bacteria from the surface-expressed enzyme library"]; and p. 65, ll. 21-22 ["a substrate can synthesized that has a fluorophore"]. *AGMs are "agarose gel microdroplets".
in a gel microdroplet	p. 63, l. 16-p. 65, l. 6 ["gel microdroplet"]; p. 67, ll. 12-22. ["the enzymatic reaction with substrate is carried out in AGM's with enclosed bacteria from the surface-expressed enzyme library."]; p. 63, l. 15 to p. 64, l. 15; and p. 67, ll. 12-22. *AGMs are agarose gel microdroplets.
wherein the substrate is fluorescent in the presence of the polypeptide having the desired activity;	p. 65, l. 8 to p. 66, l. 13. ["Identification of mutant enzymes with desirable properties such as novel substrate selectivity or remarkable catalytic activity can be achieved using substrates that change an assayable property, *i.e.* fluorescence intensity, ratio of multiple fluorophore emissions, antibody detectable structural changes *etc.*, upon catalytic action of the enzyme."]

New Claims 60-63	Support in the Specification[1]
c) screening the microdroplet with a fluorescent analyzer that detects fluorescence; and	p. 67, ll. 12-22 ["the AGM's with desired enzyme activities could be isolated by FACS or via fluorescence microscopy using a micromanipulator"]; Example 2, p. 73, ll. 27-28 ["cells having an allowable fluorescent signal"]; Example 7, especially p. 88, ll. 1-24; Example 8, pp. 91-92; and Example 9, pp. 93-94.
d) identifying clones detected as positive for fluorescence, wherein fluorescence is indicative of DNA that encodes the polypeptide having the desired activity.	p. 67, ll. 12-22. ["the AGM's with desired enzyme activities could be isolated by FACS or via fluorescence microscopy using a micromanipulator."].
61. The method of Claim 60, wherein the extremophiles are thermophiles.	p. 3, l. 24 ["isolation of thermostable variants"]; and *see also*, material incorporated by reference at p. 12, l. 5 ["hyperthermophiles"].
62. The method of Claim 60, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.	p. 3, l. 24 ["isolation of thermostable variants"]; and *see also*, material incorporated by reference at p. 12, l. 5 ["hyperthermophiles"; "microorganisms that inhabit scalding hot springs, freezing Arctic waters, sulfur-rich geothermal springs, highly saline waters or extremely acid or alkaline habitats"].
63. The method of Claim 60, wherein the DNA for generating the library is obtained using a culture-independent system.	*See* material incorporated by reference at p. 12, ll. 15 *et seq.* ["Bypassing the culture hurdle. . ."]

APPENDIX B

SOURCE	PROPOSED COUNT
Claim 60 of the present application	60. A method for identifying polypeptides having a desired activity using high throughput screening of genomic DNA comprising: a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms, wherein the organisms are extremophiles; b) enclosing a fluorescent substrate and at least one clone from the library in a gel microdroplet, wherein the substrate is fluorescent in the presence of the polypeptide having the desired activity; c) screening the microdroplet with a fluorescent analyzer that detects fluorescence; and d) identifying clones detected as positive for fluorescence, wherein fluorescence is indicative of DNA that encodes the polypeptide having the desired activity.

APPENDIX C

Comparison of the Short Patent Claims with Proposed Count

Short Claim	Count
1. A method for identifying bioactivities or biomolecules using high throughput screening of genomic DNA comprising: a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms; b) encapsulating a bioactive fluorescent substrate and at least one clone of the library in a gel microdroplet, wherein the substrate is fluorescent in the presence of a bioactivity or biomolecule; c) screening the microdroplet with a fluorescent analyzer that detects bioactive fluorescence; and d) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule.	Claim 1 is the broad genus, which encompasses the species of Claim 8 that corresponds to the Count. The method is obvious over the proposed Count in view of at least Brennan, *Chem. & Eng. News* 74:31-33 (1996) and U.S. Patent No. 6,323,030. Brennan teaches generating an expression library from a mixed population of organisms. U.S. Patent No. 6,323,030 teaches encapsulation of a clone in a gel droplet andscreening with a fluorescent analyzer (FACS) to detect cells (col. 60, l. 67 to col. 61, l. 20).

Short Claim	Count
2. The method of claim 1, wherein the bioactivity is an enzyme is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.	Claim 2 is dependent on Claim 1. Claim 2 further provides specific enzymes to be identified by the method. Claim 2 is obvious over the proposed Count given that at least Brennan suggests several of these enzymes as good targets for enzyme discovery. *Chem. & Eng. News* 74:31-33 (1996). Also U.S. Patent No. 6,323,030 cites viral proteases as one of the enzymes which can be identified (col. 7, l. 59).
3. The method of claim 1, wherein the library is generated in a prokaryotic cell.	Claim 3 is dependent on Claim 1. Claim 3 further provides for the expression library being generated in a prokaryotic cell. Claim 3 is obvious over the proposed Count given that expression libraries were commonly prepared in prokaryotic cells, such as *E. coli*. *See, e.g.*, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 17.11-17.36 (2nd ed. Cold Spring Harbor, NY, 1989). *See also* U.S. Patent No. 6,323,030, at col. 27, ll. 9-11 ("...plasmid DNA...are introduced into *E. coli*...").
4. The method of claim 3, wherein the prokaryotic cell is gram negative.	Claim 4 is dependent on Claim 3. Claim 4 further provides that the expression library be prepared in a Gram negative cell. Claim 4 is obvious over the proposed Count given that expression libraries were commonly prepared in *E. coli*, a Gram negative prokaryote. *Id.* *See also*, U.S. Patent No. 5,149,639 at col. 8 *et seq.*: "Example 5 Construction of a Genomic Library of *Streptomyces antibioticus* ATCC 11891 in pNJ1 and introduction into *E. coli*." *See also*, U.S. Patent No. 6,323,030, col. 27, ll. 9-11 ("...plasmid DNA...are introduced into *E. coli*...").

Short Claim	Count
4. The method of claim 3, wherein the prokaryotic cell is gram negative.	Claim 4 is dependent on Claim 3. Claim 4 further provides that the expression library be prepared in a Gram negative cell. Claim 4 is obvious over the proposed Count given that expression libraries were commonly prepared in *E. coli*, a Gram negative prokaryote. *Id. See also*, U.S. Patent No. 5,149,639 at col. 8 *et seq.*: "Example 5 Construction of a Genomic Library of *Streptomyces antibioticus* ATCC 11891 in pNJ1 and introduction into *E. coli*." *See also*, U.S. Patent No. 6,323,030, col. 27, ll. 9-11 ("...plasmid DNA...are introduced into *E. coli*...").
5. The method of claim 4, wherein the prokaryotic cell is *E. coli*.	Claim 5 is dependent on Claim 4. Claim 5 further provides for the expression library being prepared in *E. coli*. Claim 5 is obvious in view of the proposed Count, because expression libraries were commonly prepared in *E. coli*. *Id. See also*, Example 5 of U.S. Patent No. 5,149,639. *See also*, U.S. Patent No. 6,323,030, col. 27, ll. 9-11 ("...plasmid DNA...are introduced into *E. coli*...").

Short Claim	Count
6. The method of claim 5, wherein prior to step b), the *E. coli* is transferred to a Streptomyces sp.	Claim 6 is dependent on Claim 5. It is assumed that Claim 6 is meant to provide that the DNA expression library prepared in *E. coli* can be transferred to a Streptomyces sp. Given this assumption, Claim 6 is obvious in view of the proposed Count, because the transfer of libraries between species was known to the skilled artisan. For example, *see* Bormann *et al.*, *J. Bacteriol.* 178: 1216-8 (1996), which teaches the expression of a genomic library obtained from *S. tendae* and expressed in *S. lividans* TK23 by the use of cosmids. See also, Example 6 of U.S. Patent No. 5,149,639 which describes the transfer of the *Streptomyces antibioticus* library from *E. coli* to *Streptomyces erythreus* (col. 9, ll. 1 *et seq.*).
7. The method of claim 6, wherein the Streptomyces sp. is *Streptomyces venezuelae*.	Claim 7 is dependent on Claim 6. Thus, the assumption for Claim 6 applies for Claim 7. Therefore, Claim 7 further provides that the Streptomyces species is *Streptomyces venezuelae*. Claim 7 is obvious in view of the proposed Count, because the skilled artisan would have known that the DNA could be transferred into different *Streptomyces* species, including *S. venezuelae*. *See*, *e.g.*, U.S. Patent No. 5,149,639 at col. 14, ll. 39-47. Also, *S. venezuelae* is known to produce chloramphenicol (Sala and Westlake, *Can. J. Microbiol.* 1966 12(4): 817-29.)
8. The method of claim 1, wherein the expression library contains DNA obtained from extremophiles.	Claim 8 is dependent on Claim 1. Claim 8 with all the limitations of Claim 1 is the Count.

Short Claim	Count
9. The method of claim 8, wherein the extremophiles are thermophiles.	Claim 9 is dependent on Claim 8. Claim 9 provides that the extremophile is a thermophile. Claim 9 is obvious over the proposed Count in view of at least Brennan (1996), which suggests preparing libraries to hyperthermophiles, a species of thermophile.
10. The method of claim 9 wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.	Claim 10 is dependent on Claim 9. Claim 10 further recites selected extremeophiles. Claim 10 is obvious over the proposed Count in view of at least Brennan (1996), which suggests preparing, for example, an expression library to hyperthermophiles.
11. The method of claim 1 wherein the bioactive substrate comprises C12FDG.	Claim 11 is dependent on Claim 1. Claim 11 further provides that the detection substrate is C12-fluorescein-di-β-D-galactopyranoside (C12FDG). This was a commonly used detection substrate. *See, e.g.*, Plovins *et al.*, *Appl. Environ. Microbiol.* 60: 4638-41 (1994). Therefore, Claim 11 is obvious over the proposed Count.
12. The method of claim 1, wherein the bioactive substrate comprises a lipophilic tail.	Claim 12 is dependent on Claim 1. Claim 12 further provides that a bioactive substrate, such as C12FDG, have a lipophilic tail. Claim 12 is obvious over the proposed Count, because the skilled artisan would have known that the attachment of lipophilic carbon chains would impact the solubility of substrates, which would be an useful attribute. *See*, *e.g.*, Plovins *et al.*, *Appl. Environ. Microbiol.* 60: 4638-41 (1994), which discloses that C12FDG was a useful substrate for β-galactosidase detection by flow cytometry.

Short Claim	Count
13. The method of claim 1, wherein the samples are heated before step b).	Claim 13 is dependent on Claim 1. Claim 13 further provides the step of heating the cells to prevent or reduce extraneous activity. Claim 13 is obvious over the proposed Count because cell fixation methods (*e.g.*, heat and time, permeabilization agents, etc.) was known in the art. *See, e.g.*, R. E. Cunningham, "Flow Cytometry" in MOLECULAR BIOMETHODS HANDBOOK 653-667(Humana Press, 1998) and U.S. Patent No. 5,321,130, at col. 12, ll. 9-35, which describes that heating, *inter alia*, can be used to kill cells and allow entry of the fluorescent substrate into the cells.
14. The method of claim 11, wherein the heating is at about 70°C.	Claim 14 is dependent on Claim 11. Claim 14 further provides that the heating should be at 70°C. Claim 14 is obvious over the proposed Count because heating at 70°C is expected by the skilled artisan to kill the cells without denaturing the proteins. *See, e.g.*, U.S. Patent No. 5,321,130 at col. 12, ll. 9-35.
15. The method of claim 14, wherein the heating occurs at about 30 minutes.	Claim 15 is dependent on Claim 14. Claim 15 further provides that the heating step be performed for a period of 30 minutes. Claim 15 would have been obvious over the proposed Count, because typically heat fixation is performed for a period of time depending on the temperature to insure the death of the cell. *See, e.g.*, U.S. Patent No. 4,225,669 at col. 4, ll. 59-66 for staining periods for bacteria and *See, e.g.*, U.S. Patent No. 5,321,130 at col. 12, ll. 9-35.

Short Claim	Count
16. The method of claim 1, wherein the fluorescent analyzer comprises a FACS apparatus.	Claim 16 is dependent on Claim 1. Claim 16 further recites that the fluorescent analyzer is a FACS apparatus. Claim 16 is obvious over the proposed Count, because it was known that FACS was one of the most common high throughput methodologies available for screening cells. *See, e.g.,* Fouchet *et al.*, *Biol. Cell.* 78: 95-109 (1993) and Claim 1 of U.S. Patent No. 6,323,030.
17. The method of claim 1, wherein the expression library is biopanned before step b).	Claim 17 is dependent on Claim 1. Claim 17 further provides that the expression library is obtained by genomic biopanning. Claim 17 is obvious over the proposed Count because tagging nucleic acids with fluorescently labeled oligonucleotide probes (referred to as "biopanning") was known. *See, e.g.*, Wallner *et al.*, *Cytometry* 14: 136-143; *see also*, U.S. Patent No. 6,057,103.
18. The method of claim 1, including the additional steps of: subjecting an enzyme encoded by the DNA identified in step d) to directed evolution comprising the steps of: a) subjecting the enzyme to non-directed mutagenesis; and b) screening mutant enzymes produced in step a) for a mutant enzyme.	Claim 18 is dependent on Claim 1. Claim 18 further provides for subjecting the DNA encoding the enzyme to random mutagenesis and screening the mutants derived thereby. Claim 18 is obvious over the proposed Count, because methods of performing random mutagenesis and methods of screening the mutants were known to the skilled artisan. *See, e.g.,* James D. Watson *et al.*, eds. RECOMBINANT DNA, "In Vitro Mutagenesis," 191-202 (2nd ed., W. H. Freeman & Co., New York, 1992). Directed evolution is discussed extensively in U.S. Patent No. 6,323,030, *e.g.*, Abstract and col. 8, ll. 10-12

Short Claim	Count
19. The method of claim 1, wherein the prokaryotic expression library is normalized before step b).	Claim 19 is dependent on Claim 1. Claim 19 further provides for the DNA library to be normalized in their representation of the genome populations from the original examples. Normalization of DNA libraries was known and described in the art and thus Claim 19 is obvious over the proposed Count. *See, e.g.,* Soares *et al., Proc. Nat'l. Acad. Sci. USA* 91: 9228-32 (1994) and Patanjali *et al., Proc. Nat'l. Acad. Sci. USA* 88: 1943-7 (1991); *see also*, U.S. Patent No. 5,763,239.
20. The method of claim 1, further comprising co-encapsulating an indicator cell in step b).	Claim 20 is dependent on Claim 1. Claim 20 further provides that the cell be encapsulated with a second indicator cell. Numerous methods of encapsulating cells were known at the time. *See, e.g.,* Weaver *et al., Biotechnology* 6: 1084-9 (1988); and U.S. Patent No. 4,801,529 (Abstract).
21. The method of claim 1, wherein the library is a prokaryotic expression library.	Claim 21 is dependent on Claim 1. Claim 21 further provides that the expression library is prepared in a prokaryotic cell system. Claim 21 is obvious over the proposed Count, because expression libraries were commonly prepared in *E. coli*, which is an example of a prokaryotic cell. *See, e.g.*, Sambrook *et al.* (1989). *See also* U.S. Patent No. 6,323,030, col. 27, ll. 9-11 ("...plasmid DNA...are introduced into *E. coli*...").

Short Claim	Count
22. A method for identifying bioactivities or biomolecules using high throughput screening of prokaryotic genomic DNA in a culture-independent system comprising: a) generating a prokaryotic expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms; b) inserting a bioactive fluorescent substrate into the clones of the library, wherein the substrate is fluorescent in the presence of a bioactivity or biomolecule; c) screening the clones with a fluorescent analyzer that detects bioactive fluorescence; and d) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule.	Claim 22 is an independent claim. Claim 22 is a species of the genus of Claim 1, which provides for a method of identifying molecules using a culture-independent system. Claim 22 is obvious over the Count in view of at least Brennan (1996), which suggests that culturing can be bypassed. *See also,* Somerville *et al.*, *Applied & Enviro. Microbiol.* 55: 548-54 (1989).
23. The method of claim 22, further comprising encapsulation the clone and the bioactive substrate prior to screening.	Claim 23 is dependent on Claim 22. It is assumed that Claim 23 is meant to read "encapsulation of the clone. . .". Given this assumption, Claim 23 provides that the clone is encapsulated prior to screening. This step is part of step (b) of Claim 1, and thus is obvious in view of the proposed Count. *See,* Somerville *et al.*, *Applied & Enviro. Microbiol.* 55: 548-54 (1989). *See also* col. 60, l. 67 to col. 61, l. 20 of U.S. Patent No. 6,323,030.

APPENDIX D

Comparison of the Present Application Claims with Proposed Count

New Claim in the Present Application	Count
60. A method for identifying polypeptides having a desired activity using high throughput screening of genomic DNA comprising: a) providing an expression library containing a plurality of clones, wherein the DNA for generating the library is obtained from a mixed population of organisms, wherein the organisms are extremophiles; b) enclosing a fluorescent substrate and at least one clone from the library in a gel microdroplet, wherein the substrate is fluorescent in the presence of the polypeptide having the desired activity; c) screening the microdroplet with a fluorescent analyzer that detects fluorescence; and d) identifying clones detected as positive for fluorescence, wherein fluorescence is indicative of DNA that encodes the polypeptide having the desired activity.	Claim 60 is the Proposed Count.

New Claim in the Present Application	Count
61. The method of Claim 60, wherein the extremophiles are thermophiles.	Claim 61 is dependent on Claim 60. Claim 61 further recites a species of extremophile, *i.e.*, thermophiles. Claim 61 is obvious over the proposed Count in view of at least Brennan, *Chem. & Eng. News* 74:31-33 (1996), which suggests preparing a library to a hyperthermophile, a species of thermophile.
62. The method of Claim 60, wherein the extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidopiles.	Claim 62 is dependent on Claim 60. Claim 62 further recites species of extremophiles, including, for example, "hyperthermophiles". Claim 62 is obvious over the proposed Count in view of at least Brennan (1996), which suggests preparing expression libraries to hyperthermophiles.
63. The method of Claim 60, wherein the DNA for generating the library is obtained using a culture-independent system.	Claim 63 is dependent on Claim 60. Claim 63 further recites a method which uses a culture-independent system. Claim 63 is obvious over the proposed Count, because the skilled artisan would have appreciated the value of performing the method in a culture-independent system in view of at least Brennan (1996) ("Bypassing the culture hurdle. . . ."). *See also,* Somerville *et al.*, *Applied & Enviro. Microbiol.* 55: 548-54 (1989).